Intragenic deletions of ALDH7A1 in pyridoxine-dependent epilepsy caused by Alu-Alu recombination

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Abstract: OBJECTIVE To investigate the role of intragenic deletions of ALDH7A1 in patients with clinical and biochemical evidence of pyridoxine-dependent epilepsy but only a single identifiable mutation in ALDH7A1. METHODS We designed a custom oligonucleotide array with high-density probe coverage across the ALDH7A1 gene. We performed array comparative genomic hybridization in 6 patients with clinical and biochemical evidence of pyridoxine-dependent epilepsy but only a single detectable mutation in ALDH7A1 by sequence analysis. RESULTS We found partial deletions of ALDH7A1 in 5 of 6 patients. Breakpoint analysis reveals that the deletions are likely a result of Alu-Alu recombination in all cases. The density of Alu elements within introns of ALDH7A1 suggests susceptibility to recurrent rearrangement. CONCLUSION Patients with clinical pyridoxine-dependent epilepsy and a single identifiable mutation in ALDH7A1 warrant further investigation for copy number changes involving the ALHD7A1 gene.

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ABSTRACT

Objective: To investigate the role of intragenic deletions of *ALDH7A1* in patients with clinical and biochemical evidence of pyridoxine-dependent epilepsy but only a single identifiable mutation in *ALDH7A1*.

Methods: We designed a custom oligonucleotide array with high-density probe coverage across the *ALDH7A1* gene. We performed array comparative genomic hybridization in 6 patients with clinical and biochemical evidence of pyridoxine-dependent epilepsy but only a single detectable mutation in *ALDH7A1* by sequence analysis.

Results: We found partial deletions of *ALDH7A1* in 5 of 6 patients. Breakpoint analysis reveals that the deletions are likely a result of *Alu-Alu* recombination in all cases. The density of *Alu* elements within introns of *ALDH7A1* suggests susceptibility to recurrent rearrangement.

Conclusion: Patients with clinical pyridoxine-dependent epilepsy and a single identifiable mutation in *ALDH7A1* warrant further investigation for copy number changes involving the *ALHD7A1* gene. ***Neurology*® 2015;85:756–762**

GLOSSARY

α-AASA = α-aminoadipic semialdehyde; **CGH** = comparative genomic hybridization; **PDE** = pyridoxine-dependent epilepsy.

First described in 1954, pyridoxine-dependent epilepsy (PDE) is a metabolic epileptic encephalopathy characterized by pharmacoresistant seizures that typically come under control after initial administration followed by supplementation of pyridoxine at pharmacologic doses. The biochemical and genetic bases of this rare familial epilepsy were solved in 2006 when mutations in *ALDH7A1* resulting in dysfunction of the protein antiquitin were discovered.[1] Metabolic changes consistent with PDE can be detected by measuring elevated levels of the biomarker α-aminoadipic semialdehyde (α-AASA) in various body fluids.[1,2] As elevations of α-AASA are also present in patients with molybdenum cofactor deficiency and isolated sulfite oxidase deficiency,[3] genotyping of *ALDH7A1* is required to confirm the diagnosis. In the vast majority of published cases, homozygous or compound heterozygous mutations of both *ALDH7A1* alleles have been detected.

We investigated 6 patients with a clinical diagnosis of PDE and positive biomarkers in which only a single, heterozygous mutation in *ALDH7A1* could be identified by sequence analysis. We designed a custom oligonucleotide array that included high-density probe coverage of the *ALDH7A1* gene to look for intragenic deletions or duplications that would have been missed by conventional sequence analysis. Using this strategy, we found partial deletions of *ALDH7A1* in 5 of 6 patients, each of which is likely the result of an *Alu-Alu* recombination event. Our results suggest that in patients with clinical and biochemical evidence of PDE in the setting of a

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Table 1 *ALDH7A1* mutations in the literature

Study	No. of families	No. with both mutations identified	No. with large deletion mutation	No. with single mutation identified
Mills et al.[5]	30	27	0	3[a]
Scharer et al.[11]	15	15	0	0
Bennett et al.[4]	15[b]	14	0	1[a]
Kanno et al.[9]	4[c]	4	1 (exon 17)	0
Plecko et al.[6]	16	14	1 (exon 7)	2[d]
Mills et al.[1]	8	8	0	0
Perez et al.[8]	12	12	1 (exons 12–18)	0
Kluger et al.[7]	1	0	0	1[a]
Total	100	94	3	7[d]

[a] These cases are included in the current study and found to have a deletion as the second mutation.
[b] Three additional late-onset families with no mutations identified are not included in the table.
[c] One patient with normal pipecolic acid levels and no mutations identified is not included in the table.
[d] One of these patients was later found to have a silent *V250V* mutation.

single sequence mutation in *ALDH7A1*, studies directed at identifying intragenic deletions or duplications should be performed to identify the second mutation.

METHODS **Standard protocol approvals, registrations, and patient consents.** Patient samples and clinical information were collected after informed consent was obtained. This study was approved by ethics boards at the University of Washington and Great Ormond Street Hospital for Children, London, UK. In 5 cases, we examined DNA from the proband; in one case, we tested DNA from the father of a proband in whom a single, maternally inherited *ALDH7A1* mutation had been identified (DNA for the proband was not available). Cases 1 and 1a (sibling) were previously published[4] as K3008-3 and K3008-4; cases 2–4 were published as cases F19, F8, and F16, respectively[5]; case 5 in this study is the father of previously published[6] case 17. Case 6 was also previously reported.[7]

Array comparative genomic hybridization. We performed array comparative genomic hybridization (CGH) using a custom oligonucleotide array that had 489 probes within the *ALDH7A1* gene. All available probes in each exon as well as 5 kb upstream of the promoter were used, resulting in an average of 4 probes per exon. Average probe spacing in the introns was 1 kb; in addition, probes were placed 1 per 1 kb across the 100 kb 3′ and 5′ of the gene. We used standard PCR and Sanger sequencing to identify precise deletion breakpoints when possible.

RESULTS We identified 6 patients with a clinical and biochemical diagnosis of PDE described in the literature for which only a single mutation in *ALDH7A1* had been identified (table 1). We were able to test DNA from 5 of those patients and from the obligate carrier father of the sixth patient. We hypothesized that the second mutation in each case could be an intragenic deletion or duplication involving the *ADLH7A1* gene. We found a deletion encompassing at least one exon of the *ALDH7A1* gene in 5 of 6 cases (83%; table 2, figure 1).

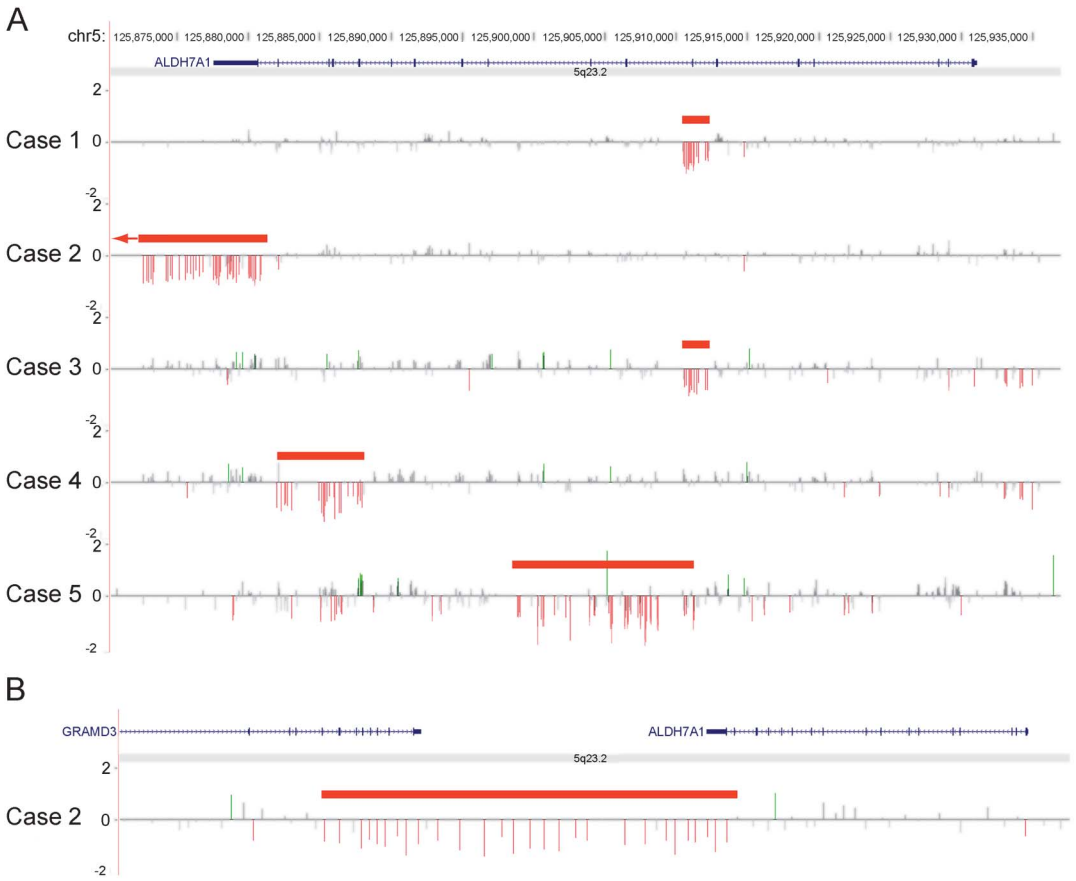
For 2 samples, we were able to perform additional studies to determine precise deletion breakpoints. Case 1 and his affected brother have a 2,366-bp deletion encompassing the entire exon 7 (45 bp) as well as ~1 kb of intronic sequence on either side of the exon (figure 2). The resulting protein is predicted to have an in-frame deletion of 15 amino acids. The

Table 2 Genotype and phenotype information for patients with heterozygous *ALDH7A1* deletions

Case	*ALDH7A1* missense	*ALDH7A1* deletion	Age at seizure onset	Initial seizures	EEG findings	Development and current status	α-AASA
1, Bennett et al.[4]	c.1197G>T (p.E399D)	Exon 7	3.5 wk	Behavioral arrest, eye aversion, perioral cyanosis	Bilateral independent onset of focal seizures, temporal regions	Persistent focal seizures; normal IQ; competitive athlete; college graduate	Plasma AASA = 3.4 μmol/L (normal <0.3)
1a (sib), Bennett et al.[4]	c.1197G>T (p.E399D)	Exon 7	9 mo	Perioral cyanosis, loss of body tone	Unk	Normal IQ; competitive athlete, attends college	Plasma AASA = 3.8 μmol/L (normal <0.3)
2, Mills et al.[5]	c.523T>G (p.W175G)	Exon 18	14 mo	Clonic, myoclonic, generalized tonic	3–4 c/s activity postcentrally, irregular fast spikes bilaterally	Normal IQ, some memory problems	Urinary AASA = 1.3 mmol/mol creatinine (normal ≤1)
3, Mills et al.[5]	c.1195G>C (p.E399Q)	Exon 7	6 d	Unk	Unk	Unk	Urinary AASA = 21 mmol/mol creatinine (normal <1)
4, Mills et al.[5]	c.866C>T (p.S289L)	Exons 14–17	5 d	Clonic, generalized tonic	L-sided epileptiform discharges	Normal development at 18 mo	Urinary AASA = 8 mmol/mol creatinine (normal ≤2.5)
5, Plecko et al.[6]	c.788G>A (p.G263E)	Exons, 8–9	Unk	Unk	Unk	Unk	Unk
6, Kluger et al.[7]	c.75insA (second mutation not found)	None detected	10 d	Prolonged tonic clonic	Focal dysrhythmias without epileptic discharge; generalized spikes	Normal IQ, attends college	Urinary AASA = 7.5 mmol/mol creatinine (normal <0.4)

Abbreviations: α-AASA = α-aminoadipic semialdehyde; Unk = unknown.

Figure 1 Intragenic deletions of *ALDH7A1*



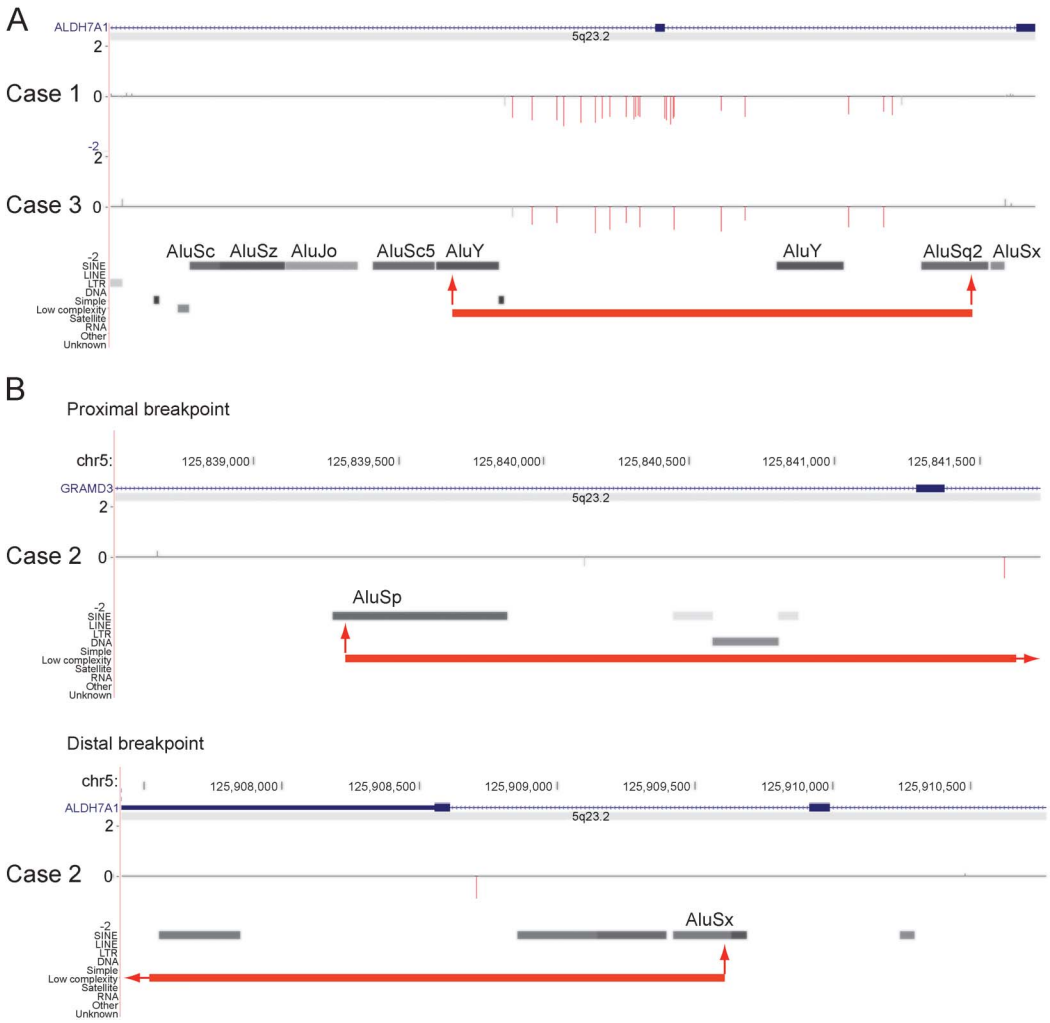
(A) Deletions in 5 unrelated individuals with pyridoxine-dependent epilepsy and a single mutation identified by Sanger sequencing. Each deletion involves at least one exon of the *ALDH7A1* gene. (B) Expanded view of the deletion in case 2, which extends proximally to the *GRAMD3* gene and is only partially represented in A.

deletion is inherited from the mother and the point mutation (c.1197G>T) from the father. We sequenced the genomic breakpoints of the deletion and found that the deletion is the result of *Alu-Alu* recombination between an *AluY* at the proximal breakpoint and an *AluSq2* at the distal breakpoint that share 82% identity across 288 bp with an 11-bp stretch of identical sequence at the breakpoint.

For case 2, array studies identified a 70,283-bp deletion encompassing the entire terminal exon 18 (55 bp) and ~1 kb of intronic sequence upstream of the exon, ~50 kb of 3′ UTR, and the last 9 exons of the *GRAMD3* gene (figure 3). We sequenced the breakpoints of the deletion and, similar to case 1, each breakpoint lies within an *Alu* element. This suggests that the deletion is the result of *Alu-Alu* recombination between an *AluSx* element at both the proximal and distal breakpoints that share 85% identity across 133 bp with an 18-bp stretch of identical sequence at the breakpoint. The predicted effect of this deletion is unclear, though it is not likely to result in a fusion protein given that *ALDH7A1* and *GRAMD3* are transcribed in opposite directions. There is no known disease associated with *GRAMD3* mutations, and the phenotype of this patient is consistent with typical PDE, so it is possible that the partial deletion of *GRAMD3* is benign. This is supported by the finding of partial *GRAMD3* deletions in the Database of Genomic Variants (http://dgv.tcag.ca/).

Array studies also identified a minimum 1.7-kb deletion encompassing the entire exon 7 (45 bp) in case 3. This deletion appears to be similar to the deletion in case 1 (figures 1 and 2). Case 4 carries a ~6 kb deletion encompassing exons 14–17, and the carrier father in case 5 has a ~10 kb deletion encompassing exons 8 and 9 (figure 3). Though we did not have adequate DNA to perform breakpoint sequencing, we find that in each of these cases, the breakpoint regions encompass a cluster of *Alu* elements (figure 3), suggesting *Alu-Alu* recombination as a likely mechanism generating each deletion. No deletions or duplications involving *ALDH7A1* were identified in case 6. While the deletion in cases 1 and 3 is predicted to cause an in-frame deletion of 15 amino acids, the deletions in the other cases are not in-frame and likely lead to premature truncation of the protein.

Figure 2 Detailed analysis of deletions in cases 1 and 2 reveals *Alu* elements at the breakpoints



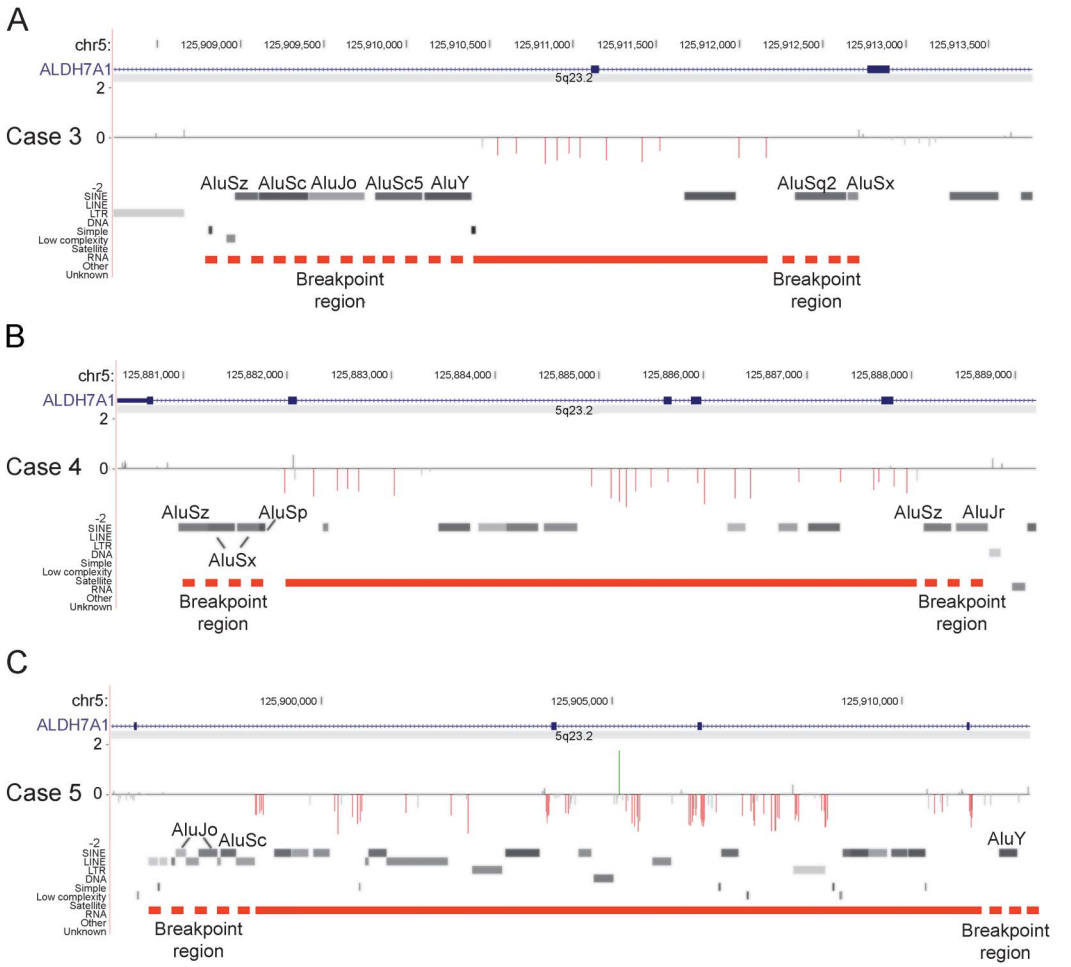
Deletion breakpoints in cases 1 (A) and 2 (B) were sequenced and found to lie within *Alu* elements. Approximate location of breakpoints is indicated by red arrows. In A, array data for case 3 are shown, but only the breakpoints of case 1 were sequenced.

We also performed array CGH for 3 individuals (K-3003-3, K-3009-3, K-3018-3) with late-onset pyridoxine-dependent seizures who had no mutations in *ALDH7A1* by sequence analysis.[4] These individuals, whose biomarkers were less conclusive, did not have any detectable deletions or duplications in the *ALDH7A1* gene. We have evaluated 138 other individuals who do not have PDE using the same array (data not shown), and none has exhibited exon-containing deletions or duplications of *ALDH7A1*. Furthermore, there are no copy number variants within the *ALDH7A1* gene reported in the Database of Genomic Variants (http://projects.tcag.ca/variation).

DISCUSSION Using a custom array, we identified heterozygous intragenic deletions in the *ALDH7A1* gene in 5 of 6 patients with PDE and positive biomarkers who had only a single mutation identified by conventional sequence analysis. The deletions range in size from 1.7 to 70 kb and encompassed 1–4 exons. In each case, the deletion breakpoints appear to lie within *Alu* elements, suggesting that aberrant recombination is the mechanism that generated the deletions. *Alu* elements are a family of short (~300 bp) interspersed repeat elements in the human genome that are remnants of once-active transposable elements. Although not identical in sequence, they are highly related to each other, which can facilitate aberrant recombination and subsequent deletion or duplication, making them an important source of mutation.

Deletions within the *ALDH7A1* gene have been reported in only 3 cases in the literature (table 1). One individual was found to have a deletion of exon 7 (case 7, c.567_611del) by sequencing cDNA. In another study, a patient with a deletion encompassing exons 12–18 was identified by CGH, though the precise genomic breakpoints were not evaluated in

Figure 3 Breakpoint regions of cases 3, 4, and 5 contain *Alu* elements



Deletion breakpoints in cases 3 (A), 4 (B), and 5 (C) are located within clusters of *Alu* elements. Deleted region in each case is indicated by the solid red bar; the dotted bar indicates the breakpoint region as determined by the region between the last deleted probe and the next probe with normal $\log_2$ ratio. In each case, the breakpoint region is within a cluster of *Alu* elements.

either case.[6,8] A third case was reported to have a deletion of exon 17 due to an *Alu-Alu* recombination event in introns 16 and 17.[9] Notably, 2 of 5 deletions in our study encompass exon 7, suggesting that this exon may be particularly susceptible to deletion. In the 2 cases where we had sufficient DNA to sequence the deletion breakpoints, we found that the breakpoints lie within *Alu* elements. Furthermore, in the remaining cases, array CGH data show that the breakpoints are also predicted to be within a cluster of *Alu* elements. The *ALDH7A1* gene is relatively *Alu*-rich, with 33% of the sequence made up of *Alu* repeats, compared to the genome average of 10%.[10]

Of 100 families studied in the literature, both mutations in *ALDH7A1* had been found in 94 families, and only a single mutation had been identified in 6 families[1,4–6,8,9,11] (table 1). Here, we identify the second mutation in 5 of the 6 unexplained families in the literature. Specialized studies to detect genomic rearrangements should be considered when only a single mutation is identified. While we selected custom CGH, other methods that might be employed in the clinical laboratory include quantitative PCR or multiplex ligation-dependent probe amplification. Notably, the deletions in 4 of our 5 cases are ~10 kb or less; therefore, detection by standard chromosome microarray would depend on the resolution of the array employed by the laboratory. cDNA sequencing could be considered if cell lines are available. We were unable to identify a second mutation in patient 6 in our study. It is possible that this patient has a mutation in regulatory or intronic sequence outside of the canonical splice site that would have been missed by prior sequencing efforts. Additional studies, such as whole-genome sequencing or RNA studies, might help identify a second mutation, though these studies were not possible due to lack of material.

More than 60 *ALDH7A1* sequence alterations have been documented in affected individuals, but

genotype–phenotype studies have failed to identify any strong genotype–phenotype correlations.[5,11,12] Patients with missense mutations that result in residual enzyme activity tend to have a milder phenotype with better developmental outcome and response to treatment.[11] Nine mutations represent 61% of disease alleles; the "common" p.E399Q (c.1195C>G) alteration represents approximately 1/3 of the mutated alleles and has been reported in both neonatal-onset and late-onset cases.[4] Indeed, in addition to the exon 7 deletion reported here, the 399 residue was also mutated in case 1 and his brother (case 1b); however, their mutation (p.E399D, c.1197G>T) is novel. Both of these siblings have had favorable developmental outcomes. Curiously, they have different phenotypes, as the older boy presented with neonatal-onset seizures (which are incompletely controlled even with antiepileptic drugs) while the younger boy's seizures did not develop until 9 months of age. To our knowledge, there are no other PDE families reported where seizures presented in affected siblings at such different ages. The mutations in case 3 are similar to those in the siblings (p.E399Q and exon 7 deletion). Age at onset in this patient was 6 days. The variable time of seizure onset in this family and another patient with similar genotype supports the suggestion that nongenetic factors may contribute to the phenotypic spectrum of PDE.[11] Genetic factors, including different second mutations in *ALDH7A1*, may also contribute. Case 2 did not present until 14 months of age, suggesting that one or both mutations in this patient may have a mild effect. The deletion in case 2 involves only the last exon of *ALDH7A1*, while the second mutation is a missense (W175G). One other patient with the W175G mutation also had a late-onset phenotype.[5] Overall, the patients in our series with one point mutation and one deletion mutation do not have markedly different phenotypes vs patients with 2 point mutations (table 2). While most patients in our series are reported to have normal IQ, detailed testing results were not available to determine whether any had specific learning deficits.

Deletions involving exon sequence within the *ALDH7A1* gene account for the majority of mutations in patients with PDE who have positive biomarkers but only one documented mutation by sequence analysis, and the density of *Alu* elements within intronic regions may facilitate intragenic deletions. These results also emphasize the clinical importance of assessing biomarkers in patients with a suspected diagnosis, even when only a single genetic mutation is found. Evaluation of exon copy number using a custom array like the one described here or another method should be implemented in the diagnostic laboratory for this purpose.

AUTHOR CONTRIBUTIONS

Heather C. Mefford: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, study supervision, obtaining funding. Matthew Zemel: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data, statistical analysis, study supervision. Eileen Geraghty: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Joseph Cook: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Peter T. Clayton: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval. Karl Paul: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. Barbara Plecko: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Philippa B. Mills: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients. Douglas R. Nordli: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. Sidney M. Gospe: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, study supervision.

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DISCLOSURE

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